Carcinogenesis in Transgenic Mouse Models

In the highly competitive world of pharmaceutical sales, what could possibly bring more than twenty pharmaceutical companies together to work cooperatively and share resources and information? Apparently, the work of the NIEHS Laboratory of Environmental Carcinogenesis and Mutagenesis (LECM) has done it. Many U.S. pharmaceutical companies are collaborating on the use of a transgenic mouse bioassay developed by the LECM to identify toxic and carcinogenic compounds.

Toxicity of pharmaceuticals, as well as other compounds to which humans are exposed, is currently identified using a two-year bioassay for carcinogenesis developed by the NTP. This bioassay, which has become the scientific standard for animal bioassays, uses B6C3F1 mice and Fischer 344/N rats of both sexes. In the traditional bioassay, animals are dosed with a test chemical for 104 weeks and then analyzed for neoplastic effects.

Transgenic mouse models developed by the LECM are genetically altered so as to be predisposed to develop tumors, but the alteration is insufficient to actually cause tumors on its own. The models overcome a major shortcoming of traditional two-year bioassays—the length of time they take to perform. Mice have a life span of approximately 2–3 years; therefore, by the time traditional bioassays are complete, the mouse subjects are reaching the end of their life span. As with all animals, the tendency to develop tumors increases with the age of the mice. "By having a short-term carcinogenesis assay, we can differentiate between age-related carcinogenesis and chemical carcinogenesis," said LECM investigator John E. French.

The LECM's transgenic mouse bioassay could cut the time for obtaining results from traditional bioassays from up to four years to one year. This could, in turn, cut the amount of time required for approval of drugs by the Food and Drug Administration, which requires toxicity testing of drugs before they can be sold to the public, resulting in large financial savings and potentially hastening a product to the market. Ray Tennant, chief of the LECM, said, "This effort by the pharmaceutical companies will provide a principle source of data that will be used to develop the test protocol [for the bioassay] and to judge the appropriate uses of the model. Because we can pool resources and evaluations, it could take as little as 1–2 years to know how well the transgenic model duplicates the two-year bioassay."

Scientists in the LECM have already shown a high concordance between results of the transgenic models and the two-year bioassay. For example, transgenic mice lacking the *p53* gene develop some of the same types of tumors and in the same sites as mice in the two-year bioassay, but they develop tumors in 6 months instead of 1.5–2 years. The NTP is interested in evaluating the transgenic model for its own purposes. "We are laying the foundation, I think, for the transition to transgenics," said Tennant. "It is inevitable that we switch to transgenics; we learn more, spend less money, and in less time. How can you beat that?"

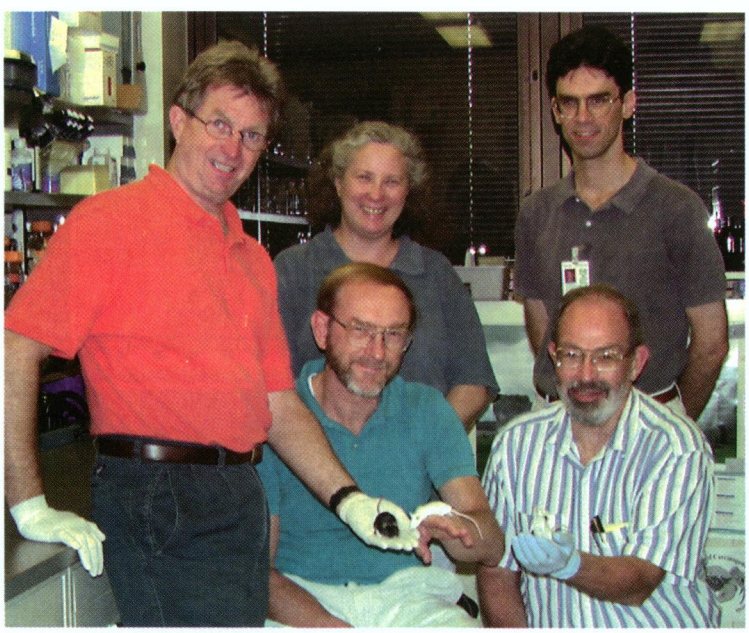
Tg.AC Model

The LECM is currently working with two transgenic models, the Tg.AC model and the *p53* model. The Tg.AC model was developed by Philip Leder at Harvard Medical School as a skin cancer model with a reporter phenotype. It carries an activated v-Ha-*ras* oncogene that alters signal transduction and growth control. The second model used by LECM is the heterozygous *p53*-deficient mouse model, which was developed by Larry Donehower and Allan Bradley at Baylor College of Medicine in Texas. This model is a strain of mice in which one of the two functioning *p53* alleles has been "knocked out." The *p53* gene is critical to a cell's response to environmental stress, including cell cycle control and DNA repair.

LECM researcher Judson Spalding is studying carcinogenesis in the Tg.AC transgenic model. Other investigators have previously shown that mutation of the cellular Ha-*ras* gene is an early event in the development of skin tumors. "We have truncated the multi-stage process by genetically initiating this early event," said Spalding. Initially, an evaluation of chemical activity via topical application in the Tg.AC model was begun by selecting chemicals with known activity in the NTP two-year bioassay. The chemicals were selected to represent a broad spectrum of genotoxic and nongenotoxic chemicals. "The agreement of chemical activity with the two-year bioassay results was high, at 88%," said Spalding. Since these studies were completed, nearly 40 other chemicals have been tested or are currently being tested with the model by the NIEHS and other researchers.

Spalding said, "We originally got into this to look at chemical carcinogenesis but we are now interested in understanding the biology of skin tumor induction in these mice in order to understand the early events involved." Spalding is most interested in the wound response. When these animals are wounded, papillomas can arise in the wound line within 4–5 weeks. "An important feature of the Tg.AC mice," he said, "is that, with the exception of the bone marrow, the transgene is not constitutively expressed in the skin or other tissues; conversely, we have never seen either spontaneous or induced tumors at any target organ site that did not express the transgene." Therefore, the transgene must be activated in the wounding process. The question is how.

One hypothesis, being explored by Spalding in collaboration with NIEHS researcher Robert Langenbach, is that inflammation is an important component of skin tumor induction. Langenbach's group is interested in the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the development of cancer.



Transgenic team. (standing l. to r.) John E. French, Carol Trempus, Mike Battalora. (seated l. to r.) Ray Tennant, Judson Spalding. (Not pictured) Ron Cannon, Randall Faircloth.

Spalding and Langenbach are trying to answer the question of whether NSAIDs inhibit the development of papillomas by inhibiting the clonal expansion of preneoplastic cells in the Tg.AC mice.

LECM researcher Ron Cannon is responsible for investigating the molecular biology of Tg.AC carcinogenesis. Cannon is interested in determining the molecular requirements for activating the Tg.AC transgene. "What I want to answer," said Cannon, "is why Tg.AC's transgene responds specifically to carcinogens and wounding." Tg.AC's transgene contains the oncogene v-Ha-*ras*, which has been integrated into the genome of the transgenic mice. The v-Ha-*ras* oncogene is under the transcriptional control of a partial zeta-globin promoter. The natural zeta-globin gene encodes the embryonic protein, which is normally expressed during days 8–15 in the blood islands of the yolk sac. This particular promoter requires an enhancer—an additional region of DNA located 40,000 base pairs upstream of the promoter and the transgene. This enhancer was not included in the Tg.AC model's transgene construct. However, the transgene is still expressed in the skin of Tg.AC mice. Cannon suggests that the transgene in the model has integrated near a region that can substitute for the required promoter control region of the transgene.

Cannon has shown with a technique called fluorescence *in situ* hybridization that the transgene localizes at a single integration site on chromosome 11 near the centromere. He has also shown that it integrates in stable tandem arrays of 4–6 copies of the transgene. Cannon searched for transcription factors known to regulate zeta-globin transcription in other tissues and then looked for those same factors in the skin of the model mice. He found that transcription factors in epithelial cells bind a factor known to be required for zeta-globin expression, a member of the family of zinc finger transcription factors called GATA. Tennant's group had previously shown by *in situ* hybridization that the transgene is activated in the cells of hair follicles. Cannon has recently shown that there is expression of GATA transcription factors in a specific group of cells associated with the hair follicle. Taken together, this implies there is a stem cell residing in the hair follicle that is the target cell at risk for tumorigenesis in Tg.AC transgenic mice.

p53 Model

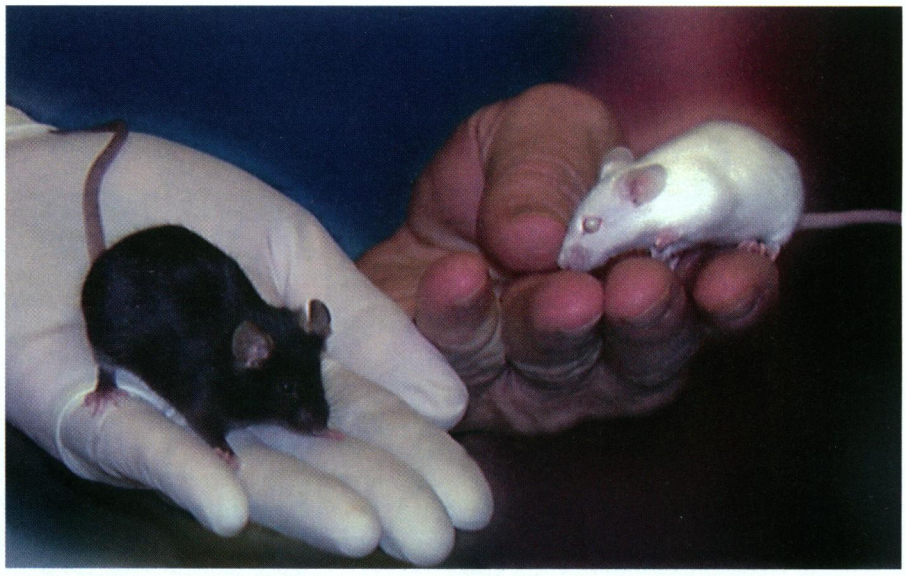
French is studying the *p53* transgenic mouse model. Current knowledge says that carcinogenic development is a multi-step process—that it requires several genetic events for cancer to develop. "The transgenic models corroborate the genetic role of proto-oncogenes and tumor suppressor genes," said French. In 1995, French and his colleagues published their results regarding the hemizygous *p53* transgenic model in the October issue of *EHP*. In this study, they looked at five chemicals: 4-vinyl-1-cyclohexene diepoxide, *p*-cresidine, *p*-anisidine, *N*-methylolacylamide, and reserpine. In all cases, the transgenic model reproduced the tumor induction seen by the NTP bioassay in number and location. "We showed reproducibility. I find that very reassuring," said French.

When French removed the tumors that developed in the transgenic mice to search for the genetic event or lesion that caused a tumor to develop, he expected to see loss of the only functional *p53* allele. Although French did find such a loss in some cases, he did not find it as often as he had anticipated. For example, when animals were treated with *p*-cresidine, an aromatic amine that causes bladder tumors, only one-fifth of the tumors induced lost the wild-type *p53* allele. In animals treated with the human carcinogen benzene, French observed a loss of the *p53* allele in two-thirds of the tumors examined.

French's group also expected to find mutations in the lone functional *p53* allele when they didn't see loss. Normally, when mutations are found in the *p53* gene, they are most often found in exons 4, 5, 6, 7, and 8. French used a technique called single strand conformational polymorphism to screen for mutations in *p53* in bladder tumors. "We seldom found mutation in exons 4–8 of *p53* in the bladder tumors," said French, "yet we can observe mutation in the bacterial *lac* neutral reporter gene, which demonstrates mutagenization of the bladder and indicates that other critical genes can be mutated." In contrast, in animals treated with phenolphthalein, a chemical recently eliminated from laxative formulations by drug manufacturers due to its carcinogenic potential, every tumor lost the whole *p53* wild-type allele. "These studies suggest that this model may be appropriate for identifying mutagenic carcinogens independent of mutations or loss of the *p53* wild-type allele," said French. "Now we have a dichotomy. There aren't many genes that are critical in cells of all types, but *p53* is involved in . . . up to 80% of cancers. The *p53*-deficient mouse is set up for chromosomal, genetic, and other critical events that drive tumorigenicity."

The *p53* gene sits at the junction of many pathways, including cell cycle events, DNA replication, and apoptosis (or programmed cell death). All of these processes are important to tumorigenesis and are currently being researched by scientists all over the world. The Tg.AC and the *p53* transgenic models developed by the LECM may offer such scientists a faster, cheaper, and easier way to answer many basic questions about tumorigenicity.

Tracy Lyon



Model mice. Transgenic mice models may one day replace traditional two-year mouse bioassays currently used to test for carcinogenesis.